

CLAIMS

- 1) An in vitro method for generating a human cell culture which comprises at least 90% human melanocytes which are capable of melanin and/or L-dopa synthesis, wherein epidermal human cells are cultured in a serum-free, pituitary extract-free and phorbol ester-free medium in the presence of antibiotics, and wherein said epidermal cells are further subcultured in the presence of at least 0,75 mM Ca^{2+} .
- 10 2) An in vitro method for generating a human cell culture which comprises at least 90% human melanocytes which are capable of melanin and/or L-dopa synthesis, comprising
 - a) mechanically and/or enzymatically separating epidermal cells from dermal cells,
 - b) culturing the separated epidermal human cells in a serum-free, pituitary extract free and phorbol ester-free medium in the presence of antibiotics, and
 - 15 c) further subculturing the epidermal cells in the presence of at least 0,75 mM Ca^{2+} .
- 3) An in vitro method for generating a human cell culture which comprises at least 90% human melanocytes which are capable of melanin and/or L-dopa synthesis, for use in an autologous cell implantation, comprising
 - a) mechanically and/or enzymatically separating the epidermal cells from the dermal cells,
 - b) culturing the separated epidermal human cells in a serum-free, pituitary extract-free and phorbol ester-free medium, in the presence of antibiotics, and
 - 20 c) subculturing the epidermal cells in the presence of at least 0,75 mM Ca^{2+} .
- 25 4) An in vitro method for generating a monoploid human cell culture which comprises at least 90% human melanocytes which are capable of melanin and/or L-dopa synthesis, comprising
 - a) mechanically and/or enzymatically separating the epidermal cells from the dermal cells
 - b) culturing the epidermal human cells in a serum-free, pituitary extract-free and phorbol ester-free medium in the presence of antibiotics, and
 - 30 c) subculturing the epidermal cells in the presence of at least 0,75 mM Ca^{2+} .
- 5) A method according to any of claims 1 to 4, wherein said human melanocyte culture is at least 90% pure, such as at least 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 % pure.
- 35 6) A method according to any of claims 1 to 4, wherein said human melanocyte culture is at least 95 to 100% pure.
- 7) A method according to any of claims 1 to 6, wherein the concentration of Ca^{2+} is at least 1 mM.
- 40 8) A method according to any of claims 1 to 6, wherein the concentration of Ca^{2+} is at least 1 to 1,6 mM.

- 9) A method according to any of claims 1 to 6, wherein the concentration of Ca^{2+} in the culture is kept at approximately 1,2 to 1,6 mM during one or more days of the cultivation period.
- 5 10) A method according to any of the preceding claims, wherein said melanocytes maintain their mitotic qualifications in the culture.
- 11) A human melanocyte culture generated by a method according to any of the preceding claims.
- 10 12) One or more cell(s) from a melanocyte culture according to claim 11, which are autologous melanocyte(s).
- 13) Two or more cells from a melanocyte culture according to claim 11, which are monoploid.
- 15 14) One or more cell(s) according to any of claims 11-13, for use as a medicament.
- 15) A composition comprising one or more cell(s) according to any of claims 11-13, for use as a medicament.
- 20 16) Use of one or more cell(s) according to any of claims 11-13, for the preparation of a pharmaceutical composition for use in an autologous cell implantation.
- 25 17) Use of one or more cell(s) according to any of claims 11-13, for the preparation of a pharmaceutical composition for the treatment of Parkinson's disease in a patient in need thereof.
- 18) A method for screening for substances capable of effecting neuronal cells from a human patient suffering from Parkinson's disease, which method comprises
 - 30 a) employing a more than 90% pure autologous human melanocyte culture from said patient
 - b) pre-plating a plate with one or more potentially effective substances,
 - c) plating one or more cell(s) from said melanocyte culture generated in step a) onto said plate,
 - 35 d) incubating said melanocytes with said substances during a decided incubation time, and
 - e) analysing the plates to identify the substances that display an effect on the plated cell(s).
- 40 19) A method according to claim 18, wherein said melanocytes are at least 90% pure, such as at least 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 % pure.
- 20) A method according to claim 18, wherein said melanocytes are at least 95 to 100% pure.

21) A method according to any of claims 18 to 20, which is executed as a high-throughput screening.

5 22) A method according to any of claims 18-21, wherein the plates in step e) are analysed on a reader such as a fluorescence-reader.

10 23) A method according to any of claims 18-21, wherein the non-viable cell(s) effected by a substance from the plate in step e), are removed, and the viable cells are analysed using a cell counter or flow cytometric analysis.

24) A method according to any of claims 18-23, wherein said melanocyte(s) used in said method is/are generated by a method according to any of claims 1-10.

15 25) Use of a method according to any of claims 18-24, for identifying a substance suitable in an individual medical treatment method for a human patient suffering from Parkinson's disease.

20 26) Use of a substance identified by a method according to any of claims 18-24 for treating Parkinson's disease in a human patient.

25 27) A method for screening for a predisposition for Parkinson's disease in a human patient, comprising testing the sensitivity of one or more cell(s) from an autologous melanocyte culture from said patient for a substance identified by a method according to any of claims 18-24.

28) A method according to claim 27, comprising a screening method according to any of claims 18-24.

30 29) A method for screening for a predisposition for Parkinson's disease in a human patient, comprising testing the sensitivity of one or more cell(s) from an autologous melanocyte culture from a patient predisposed for Parkinson's disease for a test substance, and comparing the sensitivity of said patient's one or more cell(s) from an autologous melanocyte culture, to the sensitivity of one or more cell(s) from an autologous melanocyte culture from a healthy individual.

35 30) A method for screening for substances capable of effecting neuronal cells from a human patient suffering from Parkinson's disease, which method comprises

40 a) employing a more than 90% pure autologous human melanocyte culture from each patient,

b) pre-plating a double set of plates with identical one or more potentially neurotoxic substance(s),

- c) plating one or more cell(s) from one of said melanocyte cultures generated in step a) onto each set of plates,
- d) incubating said melanocytes with said substances during a decided incubation time, and
- e) analysing the plates to identify the effect that the substances have on the plated cell(s),
5 and
- f) comparing the sensitivity of the melanocyte cultures from each patient to the substances.

31) A method according to any of claims 29-30, wherein said melanocytes are at least 90%

10 pure, such as at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% pure.

32) A method according to any of claims 29-30, wherein said melanocytes are at least 95 to 100% pure.

15 33) A method according to any of claims 29-32, wherein the plates in step e) are analysed on a reader such as a fluorescence-reader.

34) A method according to any of claims 29-32, wherein the non-viable cell(s) effected by a substance from the plate in step e), are removed, and the viable cells are analysed using a
20 cell counter or flow cytometric analysis.

35) A method according to any of claims 29-34, wherein said melanocyte(s) used in said method is/are generated by a method according to any of claims 1-10.